Journal of Molecular Structure 1241 (2021) 130566

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

Design, synthesis, antimicrobial evaluation, and molecular docking of novel chiral urea/thiourea derivatives bearing indole, benzimidazole, and benzothiazole scaffolds

Ferruh Lafzi[a], Deryanur Kilic[a,*], Melike Yildiz[b], Nurullah Saracoglu[a,*]

[a] Department of Chemistry, Faculty of Sciences, Atatürk University, Erzurum 25240, Turkey
[b] Department of Molecular Genetic and Biology, Faculty of Sciences, Atatürk University, Erzurum 25240, Turkey

ARTICLE INFO

Article history:
Received 12 February 2021
Revised 22 April 2021
Accepted 23 April 2021
Available online 7 May 2021

Keywords:
Indole
Benzimidazole
Benzothiazole
Urea/thiourea
Antimicrobial
Molecular docking

ABSTRACT

Urea/thiourea derivatives with heteroaromatic scaffolds such as indole, benzimidazole, and benzothiazole were designed, synthesized, and evaluated for their potential antimicrobial activity in vitro assays to establish against *B. cereus, S. aureus, E. coli*, and *P. aeruginosa*. Our results indicate that compounds are only active in gram-positive bacteria. Molecular docking studies were carried out for the most efficient compounds to understand the interactions with proteins involved in peptidoglycan synthesis. ADME calculations indicate that these compounds are more likely to be taken via the oral route. In summary, these findings may contribute to the design and development of candidates for more effective therapeutics in biological systems.

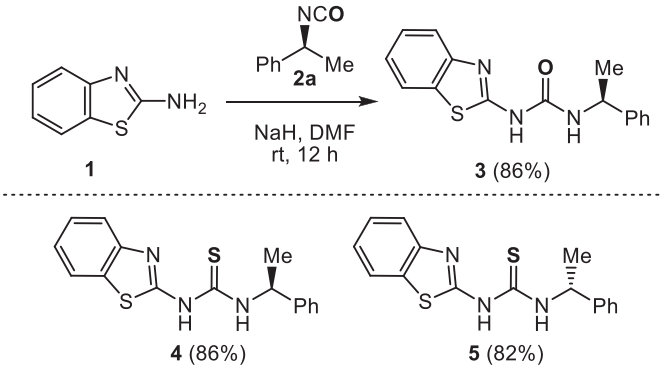
1. Introduction

Urea/thiourea subunits are valuable tool for a variety of areas ranging from chemistry to drug research. In recent years, urea/thiourea derivatives have an attracted increasing number of research groups because of their useful chemical and biological properties. Due to the acidity of the easily tunable NH and their capacity to act as H-bond donors with the respective substrates, urea and thiourea derivatives are the most commonly used groups in the design of both organocatalyst and anion receptors [1–3]. As a result of the H-bonding ability, the structurally modified (thio)ureas have also enabled many applications in the field of crystal engineering [4], supramolecular gelation, and membrane transporters for anions [5,6]. At the same time, urea and thiourea derivatives are used as the building blocks for heterocyclic ring synthesis [7]. Urea and thiourea derivatives are remarkable in medicinal chemistry, due to a wide array of their properties such as anti-HIV [8], HDL-elevating, analgesic [9], antibacterial [10–12] and antitumor [13], algaecide [14], anthelmintic [15], rodenticide [16] and plant-growth regulator [17]. They also reported to have the activities such as hypnotic [18], antifungal [19], diuretic [20], antiviral [21], anticonvulsant [22], anti-thyroidal [23], herbicidal and insecticidal [24], anti-inflammatory [25], antidepressant [26], antiulcerogenic [27], antiacetylcholinesterase [28] and antitrypanosomal [29].

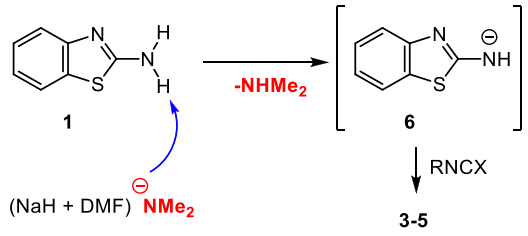
It is now important to design, and synthesis new effective antimicrobial agents since pathogens develop rapid resistance to existing medications. In this context, we designed and synthesized a series of hybrid compounds with indole, benzimidazole, and benzothiazole nucleus and chiral urea/thiourea moiety and evaluated based on their antimicrobial activity (Fig. 1). The compounds have lipophilic tails as heteroaromatic and chiral alkyl groups in *S*- and *R*-configuration. The urea and thiourea groups are the main pharmacophores which can be used as hydrogen bonding domains composed of NH groups, and oxygen and sulphur atoms acting as electron donors. Additionally, heteroaromatic rings such as indole, benzimidazole, and benzothiazole were selected based on their potential biological and therapeutic activities [30,31]. Moreover, the aforementioned scaffolds are frequently found as a key structural unit in natural products, synthetic pharmaceuticals, and agrochemicals. Also, the antimicrobial screening for synthesized compounds was performed and molecular docking analyses of the compounds were performed on two enzymes involved in peptidoglycan synthesis. To better understand the structure-activity relationship of the compounds studied, molecular docking, and ADME (absorption,

* Corresponding authors at: Department of Chemistry, Faculty of Sciences, Atatürk University, Erzurum 25240, Turkey.
E-mail addresses: deryanurerdem@atauni.edu.tr (D. Kilic), nsarac@atauni.edu.tr (N. Saracoglu).

https://doi.org/10.1016/j.molstruc.2021.130566
0022-2860/



Scheme 1. Synthesis of urea/thiourea **4** and **5**.



Scheme 2. Proposed reaction mechanism.

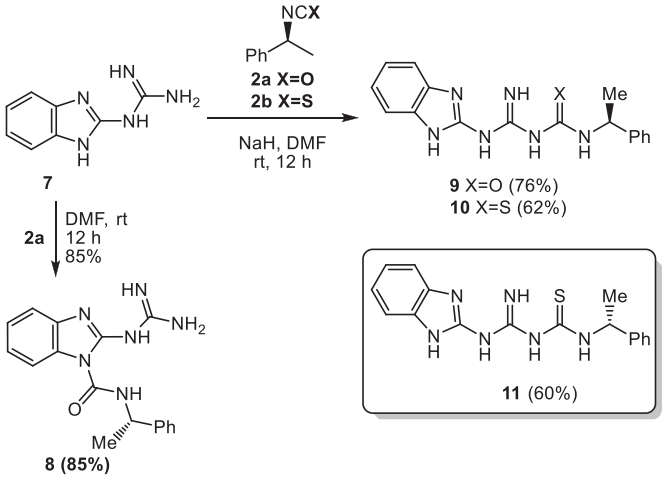
distribution, metabolism, and excretion) studies were conducted and discussed.

2. Results and discussion

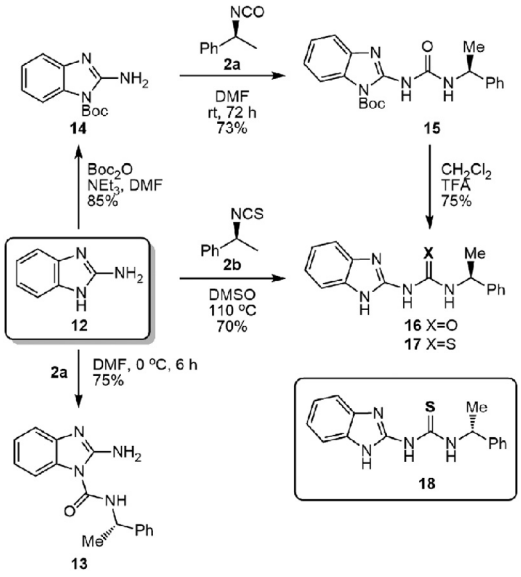
2.1. Chemistry

In our first studies on urea/thiourea synthesis, 2-aminobenzothiazole (**1**) and (*S*)-1-phenylethyl isocyanate (**2a**) were chosen as reagents to optimize the reaction conditions. The reaction in solvents such as CH_2Cl_2, THF, DMF, and DMSO at room temperature without a base did not produce the desired product **3**. However, the synthesis of benzothiazole-urea compound **3** in high yield could be achieved by utilizing sodium hydride as a base in DMF at room temperature for 12 h. The synthetic procedure adopted to obtain the target urea/thiourea derivatives **3-5** is depicted in Scheme 1. For the synthesis of thiourea enantiomeric pairs **4** and **5**, (*S*)- and (*R*)-1-phenylethyl isothiocyanates were coupled with 2-aminobenzothiazole (**1**) to afford the corresponding thioureas **4** and **5** under the same experimental conditions (Scheme 1). These results revealed that the DMF/NaH couple played a critical role in this reaction. It is well-known that NaH can combine with DMF to generate $NaNMe_2$ in situ, which is a more effective base for the deprotonation than NaH alone. We hypothesize that $NaNMe_2$ deprotonate amine group increases nucleophilicity of 2-aminobenzothiazole (**1**) (Scheme 2).

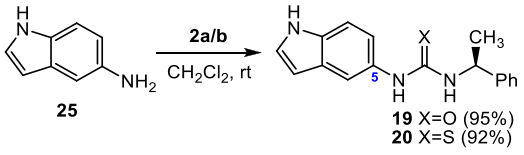
We also investigated the reactions of 2-guanidobenzimidazole (**7**) and 2-aminobenzimidazole (**12**). Both compounds were acted as ambident nucleophiles toward isocyanate **2a**. The base free-reaction of 2-guanidobenzimidazole (**7**) with and (*S*)-1-phenylethyl isocyanate (**2a**) gave the urea derivative **8** (85%), whereas the same reaction in the presence of NaH provided guanidine-isocyanate coupling to afford urea **9** in 76% (Scheme 3). In the same way, the thiourea derivatives **10** and **11** with (*S*) or (*R*) configuration were obtained in yields of 62% and 60% (Scheme 3). Treatment of 2-aminobenzimidazole (**12**) with isocyanate **2a** under the base-free conditions provided urea **13** in a 75% yield (Scheme 4). Protection of the nitrogen in the benzimidazole ring with a Boc group, using Et_3N and Boc_2O, gave regioselectively compound **14**



Scheme 3. Synthesis of urea/thiourea **8**–**11**.



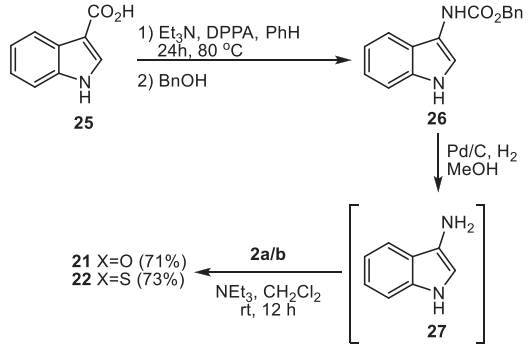
Scheme 4. Synthesis of urea/thiourea **13**, and **16**–**18**.



Scheme 5. Synthesis of urea/thiourea **19**/**20**.

(85% yield). The Boc-protected **14** was then coupled with isocyanate **2a** in NaH/DMF at room temperature and deprotected with TFA in CH_2Cl_2 to yield the urea **16**, which was distinct spectroscopic properties from **13**. Reaction of 2-aminobenzimidazole (**12**) with (*S*)-1-phenylethyl isothiocyanate and (*R*)-1-phenylethyl isothiocyanate did not produce the desired product **17** and **18** in similar reaction conditions. However, 2-aminobenzimidazole (**12**), when treated with (*S*)-1-phenylethyl isothiocyanate (**2b**) in DMSO at 100 °C, yielded the corresponding thiourea **17** in 70% (Scheme 4).

We then focused on the synthesis of C5, C3, and C2-substituted indole-based-urea/thiourea derivatives **19**-**24** (Fig. 2). The urea and thiourea groups were linked to the indole core to yield compounds **19** and **20** via a one-step procedure, without incident, as depicted in Scheme 5. A synthetic pathway to compounds **21** and **22** was planned as outlined in Scheme 6. The key step was the synthe-



Scheme 6. Synthesis of urea/thiourea **21**/**22**.

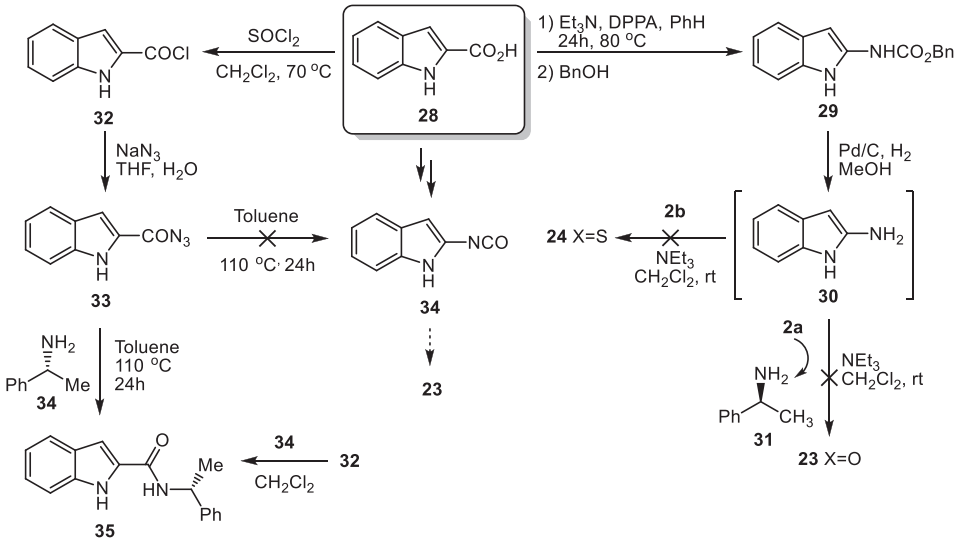
sis of intermediate **27**, by a Curtius rearrangement. In practice, the present synthesis of urea/thiourea **21** and **22** was executed straightforwardly, without any unexpected difficulties. Compounds **21** and **22** were readily prepared in three steps from commercially available indole-3-carboxylic acid (**25**) using a literature procedure [32,33] in high yields. Curtius rearrangement of indole-3-carboxylic acid (**25**) using diphenylphosphoryl azide (DPPA) and Et_3N in refluxing benzene, followed by in situ trapping with benzyl alcohol, produced the corresponding carbamate **26**. Catalytic hydrogenation of the benzyl group of **26** on Pd/C in methanol, followed by decarboxylation, yielded 3-aminoindole (**27**). Since the amine **27** has not been purified, used directly in the next step without additional purification to provide the urea/thiourea **21** (71%) and **22** (73%).

However, carbamate **29** was synthesized from indole-2-carboxylic acid (**28**) via Curtius rearrangement (Scheme 7). After catalytic hydrogenation of **29**, amine **30** was not purified. Although **30** used directly in the next step with no additional purification, this approach did not work in the synthesis of urea/thiourea **23** and **24**. In the case of (*S*)-1-phenylethyl isocyanate (**2a**), while (*S*)-1-phenylethylamine (**31**) was obtained as a meaningful product via hydrolysis of the isocyanate **2a**, the desired product **23** was not obtained. All conditions experimented upon thioisocyanate resulted in recovered starting material **2b**. After the failed synthesis via carbamate **29**, we focused our attention on the construction of the indole-isocyanate skeleton **28** with the corresponding acid **34** (Scheme 7). For this purpose, the acid **28** initially reacted with thionyl chloride to convert acyl chloride **32**. The acyl chloride **32** was treated with sodium azide in aqueous tetrahydrofuran to give acyl azide **33**, which was subjected to the thermal decomposition. Despite heating in toluene in a sealed tube at 110 °C for 24 h, the acyl azide **33** was recovered without degradation. Surprisingly, when **33** was heated in the presence of (*S*)-1-phenylethylamine (**31**) under the same reaction conditions, the amide **35** was obtained as a sole product via nucleophilic acyl substitution. To confirm the structure of **35** as a chemical, a similar reaction of acyl chloride **32** with amine **34** led to product **35**. Unfortunately, all efforts to synthesize the urea/thiourea **23** and **24** failed (Scheme 7). After this attempted synthesis, we turned our attention to synthesize their other analogs **39** and **40**. In this context, amine **38** was prepared in three-step from commercially available 2-iodoaniline (**36**), as described in the literature [34,35]. Treatment of 2-aminoindole **38** with isocyanate **2a** in THF/NaH at room temperature resulted in the desired urea **39** in a low yield (25%) (Scheme 8). When **38** and thioisocyanate **2b** were treated under the same reaction conditions, no reaction was observed and the starting materials were recovered. Using bases such as NaOMe, LDA, KF-Al_2O_3, and *n*-BuLi also did not influence the reaction. The reaction of **39** with a Lawesson's reagent did not allow to the formation of the corresponding thiourea **40**.

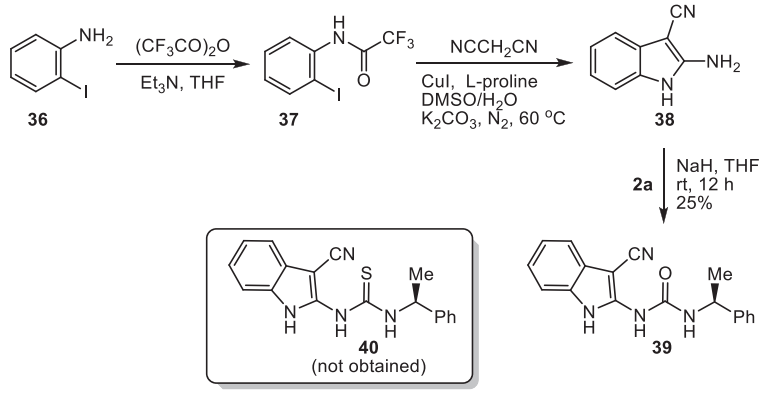
2.2. Antibacterial activity and molecular docking

Antibacterial activity of the synthesized compounds against Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) was determined using disc diffusion assay. The compounds (**8**-**11**, **13**, **16**-**18**, **20**-**21**, and **39**) indicated only an antibacterial effect against Gram-positive bacteria. Besides, a microwell dilution assay was carried out for the compounds forming the inhibition zone against *S. aureus* and *B. cereus*. The minimum inhibitory concentration (MIC) values obtained as a result of the assay were determined for all compounds and presented in Table 1. The MIC values for the compounds **9**, **16**, and **20** for both *S. aureus* and *B. cereus* were determined as 62.5 μg/mL. Also, the MIC value of compound **17** for *B. cereus* was 62.5 μg/mL. Interestingly, all compounds were effective only on Gram-positive, which may be related to the low penetration of the compounds into the outer membrane of Gram-negative bacteria.

The antibacterial effect of 2-guanidino-benzimidazole-urea derivative **9** was found to be more effective when compared with the other thiourea derivatives (**10** and **11**). In addition, the



Scheme 7. Synthesis attempts of urea/thiourea **23**/**24**.



Scheme 8. Synthesis of urea **39**.

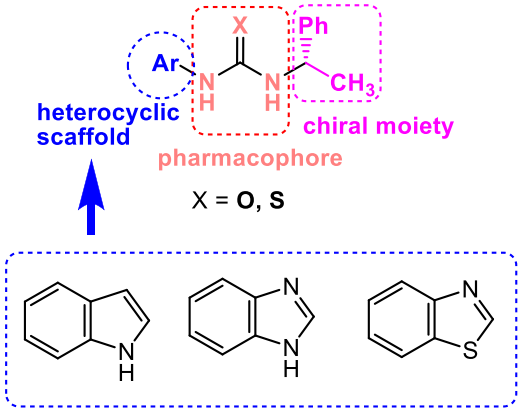


Fig. 1. Pharmacophoric features of target compounds.

thiourea derivatives (**10** and **11**) with *R*- or (*S*)-configuration exhibited similar antibacterial effects. It was determined that the benzimidazole-urea **16** and the benzimidazole-thiourea **17** with *S*-configuration significantly inhibited bacterial growth compared to the benzimidazole-thiourea **18** with *R*-configuration. The C3-substituted indole-thiourea **22** and C5-substituted indole-urea **19** showed no antibacterial effect. Moreover, the antibacterial activity of the C3-substituted indole-urea **21** and C2-substituted indole-urea compound **39** with the nitrile group was very less. However, C5-substituted indole-thiourea **20** was the most effective of the urea/thiourea derivatives bearing indole scaffolds. The benzothiazole-urea **3** and the benzothiazole-thiourea derivatives (**4** and **5**) with either *R*- or *S*-configurations had no antibacterial properties.

The *N*-substituted 2-aminobenzimidazole **13** was more effective than the *N*-substituted 2-guanidobenzimidazole **8** against Gram-positive bacteria. It was concluded that *S. aureus* was more resistant against the compounds (**13** and **17**), and *B. cereus* was more resistant against the compounds (**10**, **11**, and **21**). On the other hand, none of the substances showed any antibacterial effect on gram-negative bacteria. This selective antibacterial activity may be due to differences in the cell wall structure of Gram-negative and Gram-positive bacteria [36,37]. Similarly, some azo-based derivatives of calix[4]arene have been reported to have antibacterial activity only against Gram-positive strains [38]. However, some of the synthesized sulfanilamide-thiourea hybrids exhibited effective antibacterial activity against Gram-positive and Gram-negative bacteria [39].

Bacterial transpeptidase and transglycosylase are essential enzymes for cell wall synthesis. Consequently, the search for antibiotics targeting them nowadays continues [40]. In this study, the selective behavior of the synthesized compounds suggests that the peptidoglycan synthesis process is disrupted. Therefore, the interaction between the most active synthesized compounds and these two enzymes that play an important role in peptidoglycan synthesis has been determined through molecular docking studies. The crystal structures of *S. aureus* membrane-bound transglycosylase in complex with moenomycin (PDB ID: 3VMR) and *S. aureus* penicillin-binding protein 3 (PBP3) in complex with cefotaxime (PDB ID: 3VSL) were selected from the protein database for *in silico* studies [40,41]. In our current study, molecular docking was accomplished by adding the compounds into the binding site of moenomycin for transglycosylase and cefotaxime for the transpeptidation domain to recognize the possible binding mode and key active site interactions. While moenomycin is known as a natural occurring product that directly inhibits the function of transglycosylase, cefotaxime is known as an antibiotic that inhibits transpeptidase activity. Therefore, moenomycin and cefotaxime were used as reference molecules for the docking study. The results of the docking studies for compounds with antimicrobial effect (**9**, **16**, and **20**) were compared to reference molecules.

When the docking results of moenomycin, **9**, **16**, and **20** molecules were examined, it was determined that the moenomycin created a hydrogen bonds interaction with the Val 223, Asn 141, Lys 140, and Asp 127 residue. Compound **9** formed hydrogen bonds with the Val 223, Asn 141, Lys 140, and Asn 224 residue and pication interaction with Arg 148. The compound **16** formed hydrogen bonds with the Val 223, Asn 141, and Lys 140. Lastly, compound **20** made hydrogen bonds with the Asn 141, and Lys 140.

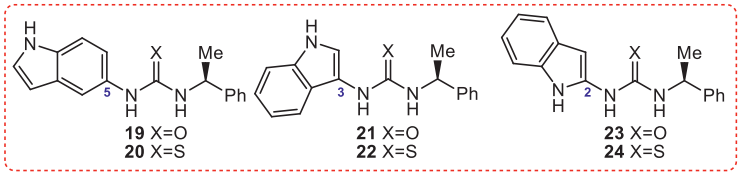
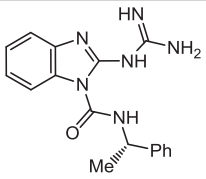
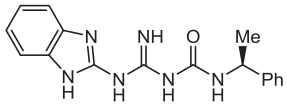
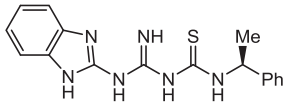
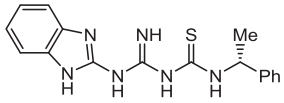
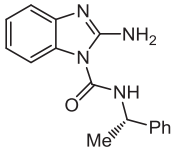
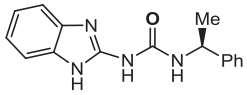
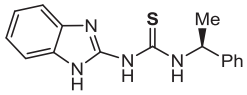
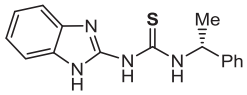
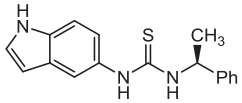
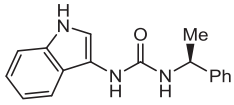
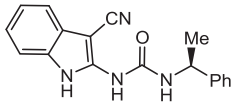
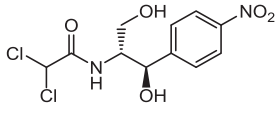


Fig. 2. Structures of target urea/thiourea **19**–**24** containing an indole ring.

Table 1
The MICs of the most active compounds in µg/mL.

Compound no	Structure		Antibacterial Activity MIC in µg/mL Gram-positive		Gram-negative	
			S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
8		>1000	>1000	-	-	
9		62.5	62.5	-	-	
10		>1000	-	-	-	
11		>1000	-	-	-	
13		500	250	-	-	
16		62.5	62.5	-	-	
17		250	62.5	-	-	
18		>1000	>1000	-	-	
20		62.5	62.5	-	-	
21		>1000	-	-	-	
39		>1000	>1000	-	-	
Chl		7.8	7.8	-	-	

Chl: Chloramphenicol, (-): Not determined

The residues Ser 227, Asn 224, Asn 141, Gln 137, Ser 132, Gln 129, Thr 122, Asn 185, Asn 179, Asn 146, Ser 149, and Gln 136 made polar interactions with moenomycin, while the residues Tyr 181, Ile 180, Pro 226, Ala 225, Val 223, Tyr 176, Val 128, Ala 118, Tyr 187, Leu 119, Val 228 and Val 138 created hydrophobic interactions. Furthermore, in the moenomycin binding site, positive and negative charge interactions were found with Arg 126, Lys 153, Arg 152, Lys 140, Arg 148, and Asp 127, Glu 100, Glu 156, Asp 147, Asp 145, Asp 184 residues, respectively.

The residues Ser 227, Asn 224, Asn 141, Gln 137, Ser 132, Gln 129, and Thr 122 procuded polar interactions with compound **9**, while the residues Tyr 181, Ile 180, Pro 226, Ala 225, Val 223, Tyr 176, Val 128, Ala 118, Tyr 187 and Leu 119 formed hydrophobic interactions. In addition, in the binding site, positive and negative charge interactions were determined with Lys 140, Arg 148, Lys 153 and Asp 127, Asp 145, and Glu 100 residues, respectively.

The residues Ser 227, Asn 224, Asn 141, Gln 137, Ser 132, Gln 129 Thr 122 and Asn 179 created polar interactions with com-

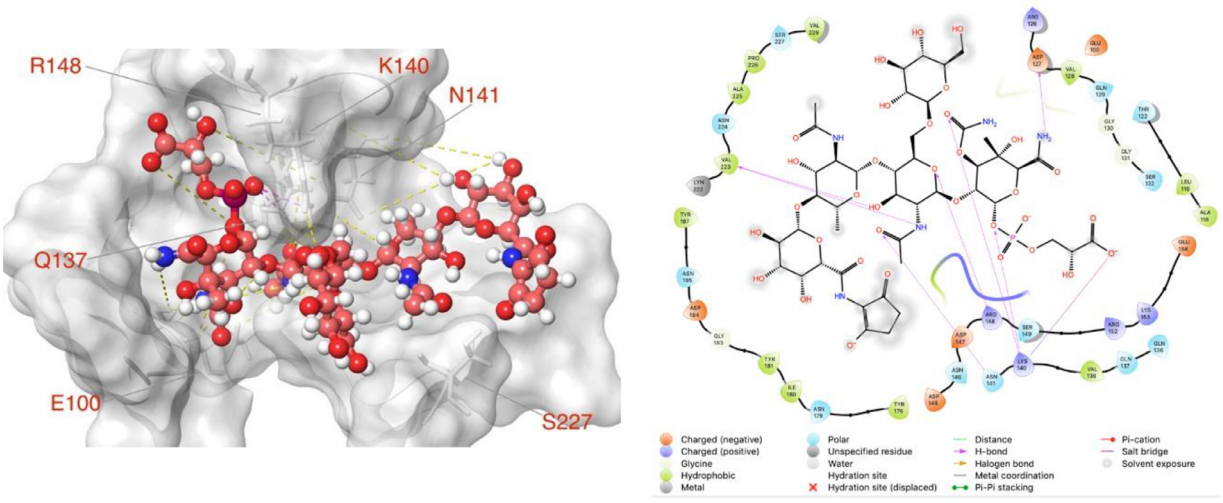


Fig. 3. 2D and 3D docking pose showing interaction for the compound MOE in the binding site of *Staphylococcus aureus* membrane-bound transglycosylase crystal structure (PDB ID:3VMR).

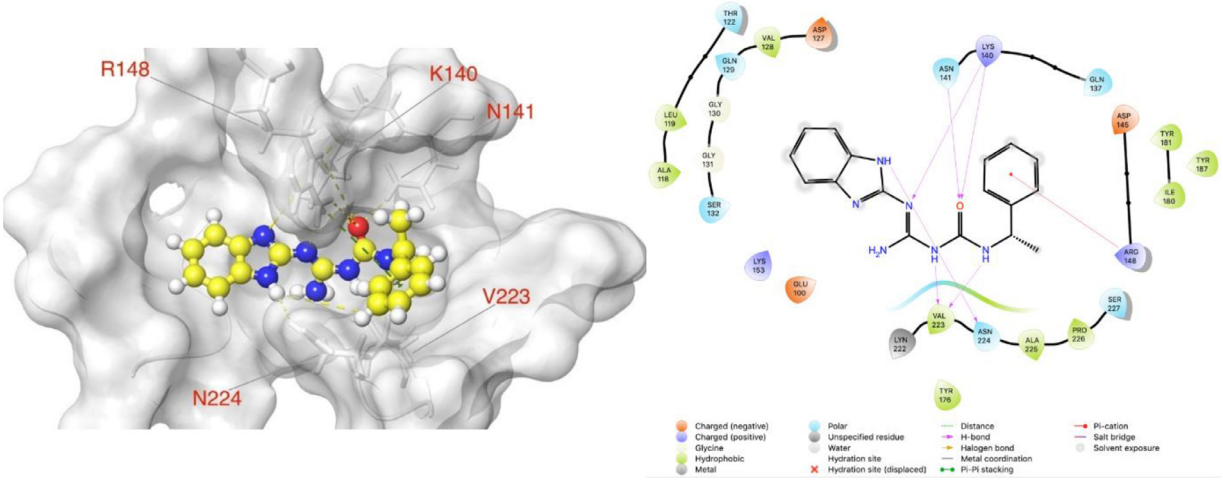


Fig. 4. 2D and 3D docking pose showing interaction for the compound **9** in the binding site of *Staphylococcus aureus* membrane-bound transglycosylase crystal structure (PDB ID:3VMR).

pound **16**, while the residues Tyr 181, Ile 180, Pro 226, Val 223, Tyr 176, Val128, Tyr 187, Met 99 and Tyr 144 made hydrophobic interactions. Additionally, in the binding site, positive and negative charge interactions were found with Lys 140, Arg 148, and Asp 127, Glu 100, Asp 145 residues, respectively.

The residues Asn 224, Asn 141, Gln137, Ser 132, Gln 129, Asn 179, and Asn 185 formed polar interactions with compound **20**, while the residues Tyr 181, Ile 180, Pro 226, Val 223, Tyr 176, Tyr 187, Tyr 142, Val 138 and Ile 76 caused hydrophobic interactions. Also, in the binding site, positive and negative charge interactions were determined with Arg 148, Lys 140, and Asp 145, Glu 100 residues, respectively.

These docking results revealed that the three compounds (**9**, **16**, and **20**) interacted with several common residues (Ser 227, Asn 224, Asn 141, Gln 137, Ser 132, Gln 129 and Thr 122, Tyr 181, Ile 180, Pro 226, Val 223, Tyr 176) when compared to the reference molecule. As a result, when the bonds and interactions made by the reference molecule (moenomycin) and the compounds in the transglycosylase inhibitor binding site are compared, no significant difference was observed in terms of the bonds formed. But it was detected that the polar interactions of the compounds were much lower than the reference molecule. Figs. 3–6 represents the docked poses of moenomycin, **9**, **16**, and **20** compounds in the transglycosylase as two and three dimensions, respectively.

In the covalent docking study, the bindings are as follows. Cefotaxime formed hydrogen bonds interaction with Ser 448, Thr 621, Thr 603, Gln 524 residues, and covalent bonding with Ser 392. Compound **9** created hydrogen bonds with the Ser 448, Thr 621 residues, and covalent bonding with Ser 392. Compound **16** formed hydrogen bonds with the Thr 621 and covalent bonding with Ser 392. Lastly, compound **20** made hydrogen bonds with the Asn 450, Ser 448, and covalent bonding with Ser 392.

The residues Thr 621, Ser 392, Gln 524, Ser 429, Asn 450, Ser 448, Thr 603, and Thr 619 had polar interactions with cefotaxime, whereas the residues Pro 660, Pro 659, Val 632, and Tyr 430 created hydrophobic interactions. Additionally, positive and negative charge interactions were found with Lys 618, Lys 395, and Glu 623 residues, in the cefotaxime-binding site, respectively.

The residues Thr 621, Ser 392, Gln 524, Ser 429, Asn 450, and Ser 448 formed polar interactions with compound **9**, while the residues Pro 660, Ala 622, Ile 522, Phe 454, and Tyr 430 produced hydrophobic interactions. In addition, in the binding site, positive and negative charge interactions were determined with Lys 395 and Glu 623 residues, respectively.

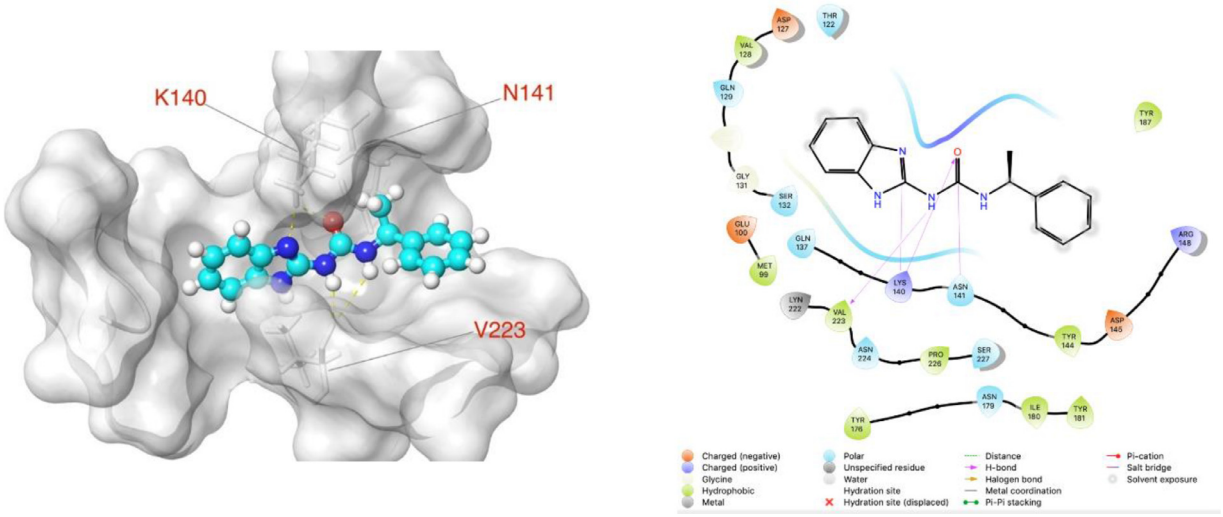


Fig. 5. 2D and 3D docking pose showing interaction for the compound **16** in the binding site of *Staphylococcus aureus* membrane-bound transglycosylase crystal structure (PDB ID:3VMR).

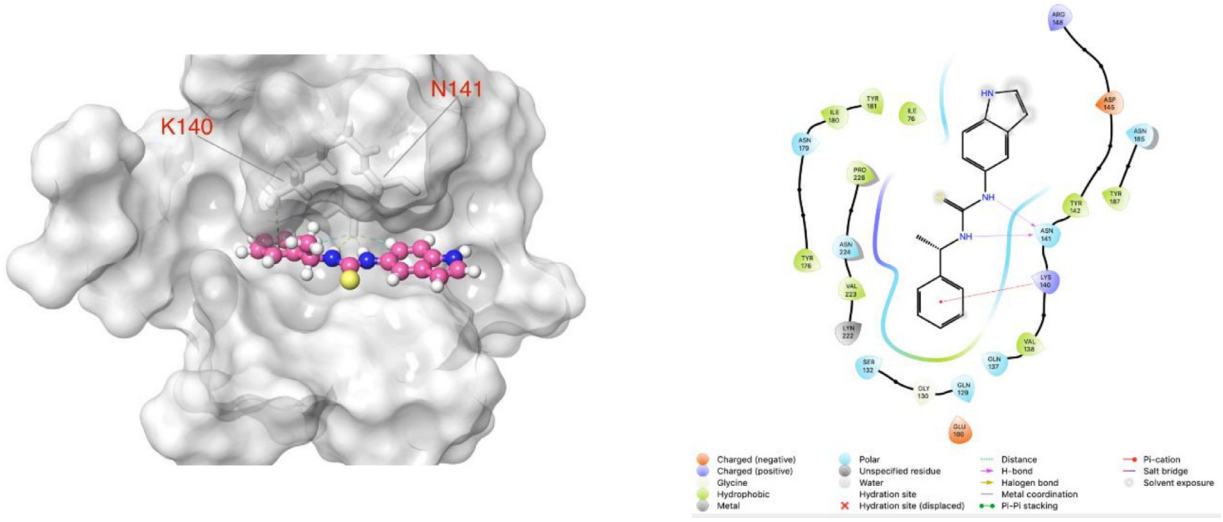


Fig. 6. 2D and 3D docking pose showing interaction for the compound **20** in the binding site of *Staphylococcus aureus* membrane-bound transglycosylase crystal structure (PDB ID:3VMR).

The residues Thr 621, Ser 392, Gln 524, Ser 429, Asn 450, Ser 448, Thr 619, and Thr 603 caused polar interactions with compound **16**, while the residues Pro 660, Ala 622, Ile 522, and Tyr 430 formed hydrophobic interactions. Also, in the binding site, positive and negative charge interactions were found with Lys 618, Lys 395, and Glu 623 residues, respectively.

The residues Thr 621, Ser 392, Gln 524, Ser 429, Asn 450, Ser 448, Thr 603, and Thr 619 made polar interactions with compound **20**, whereas the residues Pro 660, Val 632, Tyr 430, Ile 522, and Ala 622 formed hydrophobic interactions. Also, in the binding site, positive and negative charge interactions were determined with Lys 618, Lys 395, Arg 428, and Glu 623 residues, respectively. Figs. 7–10 represent the docked poses of cefotaxime, **9**, **16**, and **20** compounds in the transpeptidase as two and three dimensions, respectively.

When covalent docking results of the reference molecule (cefotaxime) and the compounds in the transpeptidase inhibitor binding site were examined, no significant difference was observed in terms of the bonds formed. Besides, it should be noted that the compounds are capable of covalent docking with the Ser 392 residue.

2.3. In silico *ADME*

The ability to absorb quickly and completely in the gastrointestinal tract, to be metabolized in a way that does not eliminate its activity immediately, and to be eliminated properly without causing any harm, are the ideal properties for oral drugs. Pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADME), which can vary depending on the chemical properties of the molecule, are important for the usability of the compounds in human therapy [42].

ADME calculations of the compounds were done using QikProp and presented in Table 2. The #stars indicate the number of properties or identifiers outside the 95% range of similar values for known drugs. The high number of stars in molecules indicates that it is less drug-like. The number of stars was 0 for all the compounds. In the Lipinski rules, which also take into account the hydrogen bond donor-hydrogen bond acceptor atoms, molecular mass, and octanol-water distribution coefficients, no deviation was observed for all compounds. Also, the number of deviations from the Jorgensen rule for these compounds was calculated as 0. These

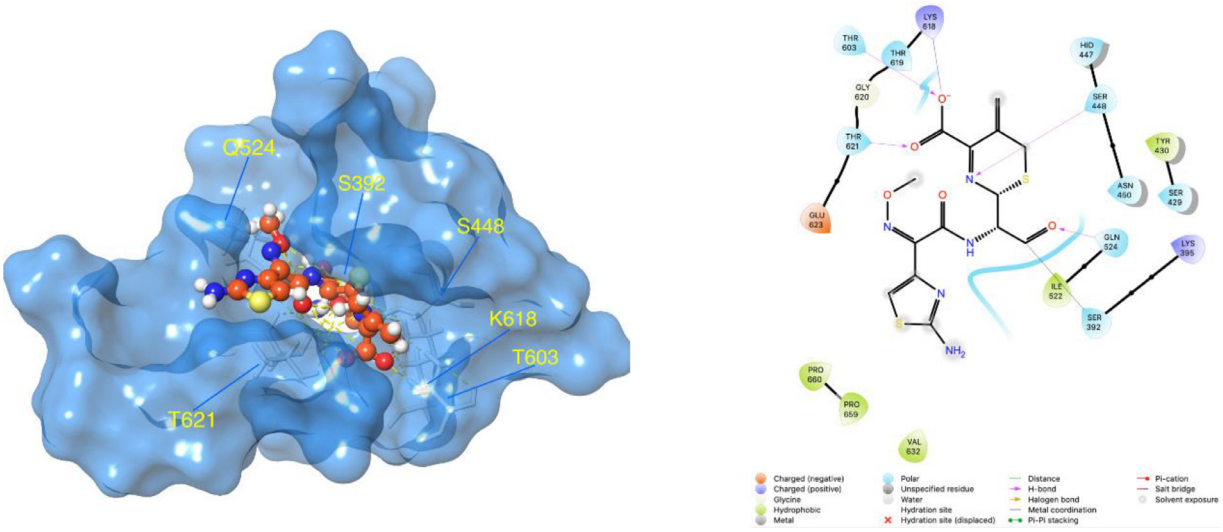


Fig. 7. 2D and 3D docking pose showing interaction for the Cefotaxime in the active site of *Staphylococcus aureus* transpeptidase domain crystal structure (PDB ID:3VSL).

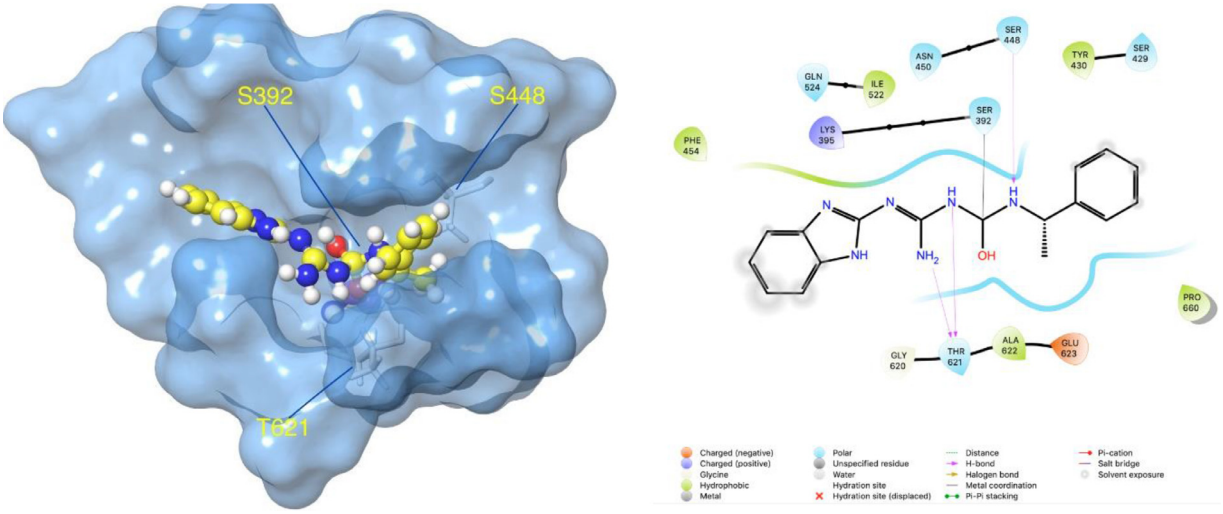


Fig. 8. 2D and 3D docking pose showing interaction for the compound **9** in the active site of *Staphylococcus aureus* transpeptidase domain crystal structure (PDB ID:3VSL).

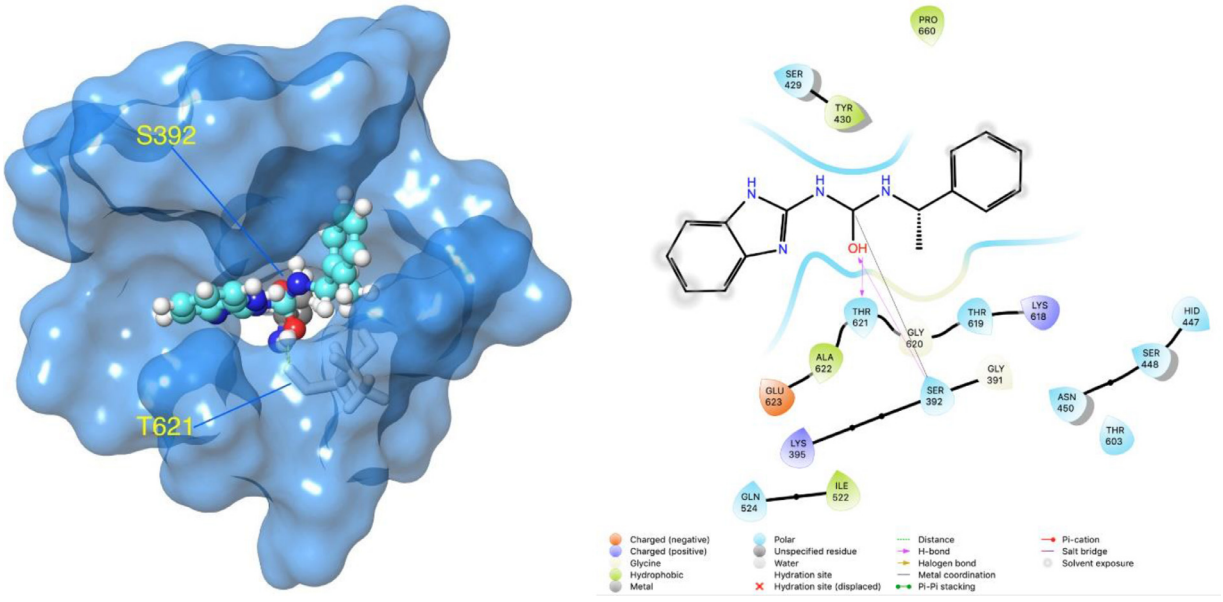


Fig. 9. 2D and 3D docking pose showing interaction for the compound **16** in the active site of *Staphylococcus aureus* transpeptidase crystal structure (PDB ID:3VSL).

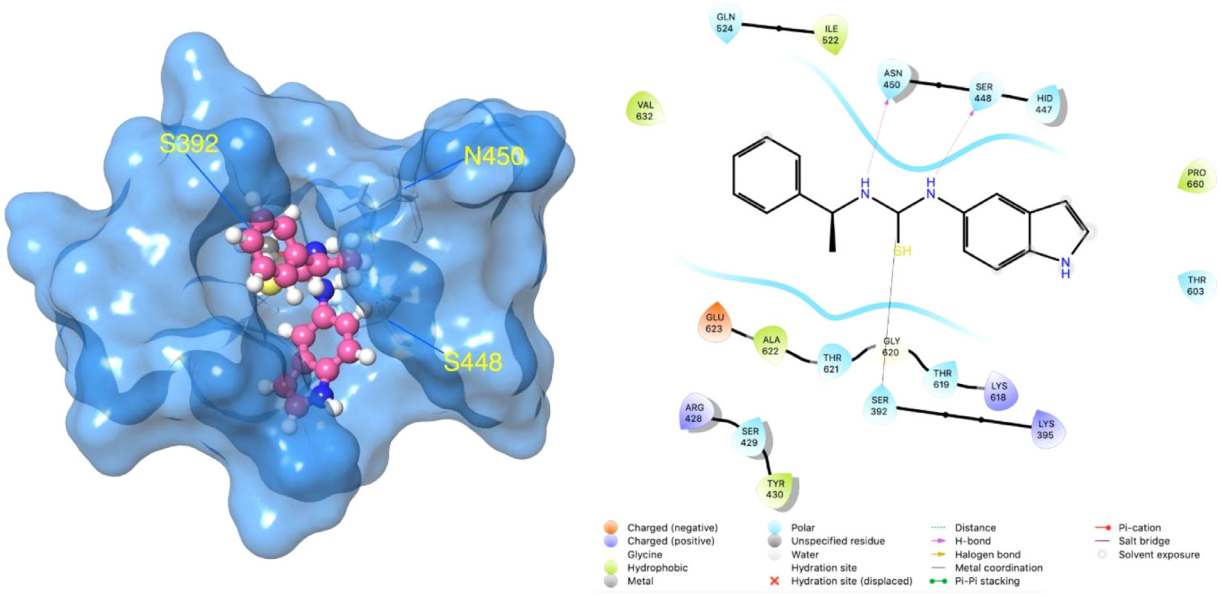


Fig. 10. 2D and 3D docking pose showing interaction for the compound **20** in the active site of *Staphylococcus aureus* transpeptidase crystal structure (PDB ID:3VSL)

Table 2
ADME prediction within Qikprop.

Compounds Acceptable range	#stars 0	QPlogBB (−3.0 to 1.2)	QPlogPo/w (−2.0 to 6.5)	QPlogHERG > -5	QPPCaco <25 poor, >500 great	QPPMDCK <25 poor, >500 great	QPlogKp (−8.0 to −1.0)	QPlogKhsa −1.5 to 1.5	Percent of Human Oral Absorption >80% is high <25% is poor	Rule of Five (Lipinski rule) max is 4	Rule of Three (Jorgensen rule) max is 3
3	0	−105	3.287	−4.720	1705.809	2514.070	−1.083	64	100.000	0	0
4	0	234	4.257	−6.224	4236.346	8089.655	−668	382	100.000	0	0
5	0	185	4.227	−6.187	3843.097	7023.390	−758	388	100.000	0	0
8	0	−1.427	1.361	−4.574	133.253	86.496	−3.253	−354	72.940	0	0
9	0	−1.356	2.530	−5.189	199.184	133.285	−2.785	23	82.911	0	0
10	0	−1.318	2.842	−6.840	324.612	291.866	−2.628	78	88.534	0	0
11	0	−1.317	2.841	−6.839	324.471	291.644	−2.629	78	88.527	0	0
13	0	−583	2.669	−4.053	655.255	471.332	−2.002	33	92.984	0	0
16	0	−534	3.115	−4.686	837.485	630.757	−1.665	163	100.000	0	0
17	0	−249	3.497	−6.203	1902.375	1825.422	−1.331	243	100.000	0	0
18	0	−225	3.495	−6.161	1986.467	1916.486	−1.299	237	100.000	0	0
19	0	−342	3.259	−4.768	1354.009	1001.998	−1.228	126	100.000	0	0
20	0	−12	4.230	−6.184	3162.658	3101.947	−830	461	100.000	0	0
21	0	−296	3.266	−4.641	1458.417	1089.459	−1.171	121	100.000	0	0
22	0	−38	4.056	−5.700	2820.325	2690.960	−1.018	423	100.000	0	0
39	0	−994	3.310	−4.870	366.393	260.413	−2.263	314	92.216	0	0
Chl	0	−1.462	1.095	−2.886	70.223	180.003	4.368	−821	66.406	0	0

results indicate that the compounds are more likely to be taken orally.

The compounds have QikProp parameters within the acceptable range, except for (QPlogHERG, QPlogBB, QPlogKp, and QPlogKhsa) parameters. The factors such as blood-brain barrier (BBB), permeability (apparent Caco-2 permeability, apparent MDCK permeability, logKp for skin permeability), and volume of distribution and binding to plasma proteins (logKhsa for serum protein binding) are associated with the distribution of compounds in the human body. The calculations have shown that **8**-**11**compounds and reference compound (Chloramphenicol) have the ability of BBB penetrate, while the other compounds do not have the ability of BBB penetrate. As is known, not all compounds can penetrate the blood-brain barrier, which ensures that these molecules have low or no central nervous system side effects. It is seen that the predicted central nervous system activity values of all the compounds are inactive or low. The logKp values representing the predicted skin permeability are in the range (-8.0 – -1.0), excluding compounds **4**-**5**, **20**, and reference compound. The degree of binding of a drug to proteins in the blood plasma can affect the effectiveness of the drug [43]. When the QPlogKhsa values obtained are analyzed, it is observed that all of the compounds have high prediction values for binding to human serum albumin. This may cause the amount of these molecules to decrease in the bloodstream. These results will help to design and develop more effective drug candidates in the biological system.

3. Conclusion

In the present study, eleven novel chiral urea/thiourea derivatives bearing indole, benzimidazole, and benzothiazole scaffolds have been designed, synthesized, and screened for their antimicrobial properties. It was found that all synthesized compounds indicated the antibacterial effect only against *S. aureus* and *B. cereus*. Besides, the docking results revealed that these compounds are predicted to have antimicrobial effects by disrupting the process of peptidoglycan synthesis, which will lead to the idea of designing new derivatives that contain groups capable of more polar interactions and covalently bond with the serine amino acid.

4. Experimental section: materials and methods

4.1. General information

All reagents and solvents were purchased from commercial suppliers and used without further purification. Column chromatography and thin-layer chromatography (TLC) were performed using silica gel 60 (70–230 Fluka) and silica gel 60 HF_{254} (Fluka), respectively. Melting points were carried out on a Buchi 539 capillary melting apparatus and uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 (100)-MHz Varian and Bruker spectrometer and are reported in δ units with $SiMe_4$ as the internal standard. Enantiomeric purity for enantioselective syntheses was determined by chiral HPLC (Hewlett Packard 1200) analysis using an enantiopure stationary phase (Daicel Chiralcel OD, OJ), eluting with i-PrOH/hexane, and using UV detection at 254 nm.

4.2. Syntheses

4.2.1. (S)-1-(Benzo[d]thiazol-2-yl)-3-(1-phenylethyl)urea (**3**)

To a stirred solution of benzo[*d*]thiazol-2-amine (500 mg, 3.33 mmol) in DMF (10 mL) NaH (55-60%) (133 mg, 3.33 mmol) was added to the solution, followed by (*S*)-(1-isocyanatoethyl)benzene (490 mg, 3.33 mmol). The reaction was stirred for 12 h at room temperature. The reaction mixture was quenched with water and then extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/ EtOAc = 7:3) to give compound 3 as a light yellow solid (225 mg, 95%) (Mp: 139-140 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.12 (bs, 1H), 7.73 – 7.70 (m, 2H), 7.40 – 7.21 (m, 7H), 5.08 (p, J = 7.0 Hz, 1H), 1.56 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8, 154.1, 143.5 (2C), 130.9, 128.7, 127.3, 126.1, 125.9, 123.4, 121.3, 120.0, 50.2, 23.0. HPLC (OJ column; hexane/2-propanol = 75/25; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 11.9 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

4.2.2. (S)-1-(Benzo[d]thiazol-2-yl)-3-(1-phenylethyl)thiourea (**4**)

Prepared according to the synthesis procedure of 3 using benzo[*d*]thiazol-2-amine (500 mg, 3.33 mmol) and (*S*)-(1-isothiocyanatoethyl)benzene (543 mg, 3.33 mmol). Purification was carried out by column chromatography using silica gel (Hexane/ EtOAc = 7:3) to afford the title compound 4 (850 mg, 86%) as a light yellow solid (Mp: 194-195 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.73 (bs, 1H), 10.84 (bs, 1H), 7.71 – 7.64 (m, 2H), 7.46 (d, J = 7.4 Hz, 2H), 7.44 – 7.35 (m, 3H), 7.34 – 7.24 (m, 2H), 5.69 (p, J=6.9 Hz, 1H), 1.73 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.0, 160.5, 142.3 (2C), 129.8, 128.8, 127.5, 126.5, 126.3, 124.4, 121.3, 120.7, 55.3, 22.3. HPLC (OJ column; hexane/2-propanol = 75/25; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 10.2 min. Enantiomeric rate: (+) = 100.0 and (-) = 0.0.

4.2.3. (R)-1-(Benzo[d]thiazol-2-yl)-3-(1-phenylethyl)thiourea (**5**)

Prepared according to the synthesis procedure of 3 using benzo[*d*]thiazol-2-amine (200 mg, 1.33 mmol) and (*R*)-(1-isothiocyanatoethyl)benzene (2217 mg, 1.33 mmol). Purification was carried out by column chromatography using silica gel (Hexane/ EtOAc = 7:3) to afford the title compound 5 (480 mg, 82%) as a light yellow solid (Mp: 194-195 °C). (*R*)-1-(Benzo[*d*]thiazol-2-yl)-3-(1-phenylethyl)thiourea (**5**) have similar experimental and spectral data with (*S*)-1-(benzo[*d*]thiazol-2-yl)-3-(1-phenylethyl)thiourea (**4**).

4.2.4. (S)-2-Guanidino-N-(1-phenylethyl)-1H-benzo[d]imidazole-1-carboxamide (**8**)

To a stirred solution of 1-(1*H*-benzo[*d*]imidazol-2-yl)guanidine (500 mg, 2.85 mmol) in DMF (10 mL) was added (*S*)-(1-isocyanatoethyl)benzene (420 mg, 2.85 mmol) and stirred at room temperature for 12 h. After the reaction is over (monitored by TLC) water (20 mL) was added and the mixture extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH = 9:1) to give compound 8 as an off-white solid (790 mg, 85%) (Mp: 88-89 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.91 (d, J = 6.5 Hz, 1H), 8.19 (d, J = 7.8 Hz, 1H), 7.44 – 7.35 (m, 5H), 7.29 – 7.24 (m, 1H), 7.23 – 7.10 (m, 2H), 5.97 (bs, 3H), 5.11 (p, J = 6.8 Hz, 1H), 1.63 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.7, 155.2, 152.4, 143.9, 140.5, 131.3, 128.8, 127.4, 126.0, 123.2, 122.1, 116.3, 115.0, 50.3, 23.0. HPLC (OJ column; hexane/2-propanol = 80/20; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 33.8 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

4.2.5. (S)-N-[N-(1H-benzimidazol-2-yl)carbamimidoyl]-N'-[1-phenylethyl]urea (**9**)

Prepared according to the synthesis procedure of **3** using 1-(1*H*-benzo[*d*]imidazol-2-yl)guanidine (500 mg, 2.85 mmol) and (*S*)-(1-isocyanatoethyl)benzene (420 mg, 2.85 mmol). Purification was carried out by column chromatography using silica gel (CH_2Cl_2/MeOH = 8:2) to afford the title compound 9 (700 mg, 76%) as an off-white solid (Mp: 127-128 °C). ^{1}H NMR (400 MHz, Acetone-d_6): δ 9.66 (bs, 2H), 8.21 (bs, 1H), 7.9 (bs, 1H), 7.44 – 7.22 (m, 7H), 7.04 (dd, J = 5.8, 3.2 Hz, 2H), 5.03 (p, J = 7.0 Hz, 1H), 1.52 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 155.7 (2C), 155.1, 145.5 (2C), 129.3 (2C), 127.8, 126.8 (2C), 121.4 (2C), 50.3, 23.2.

4.2.6. (S)-N-[N-(1H-benzimidazol-2-yl)carbamimidoyl]-N'-[1-phenylethyl]thiourea (**10**)

Prepared according to the synthesis procedure of **3** using 1-(1*H*-benzo[*d*]imidazol-2-yl)guanidine (500 mg, 2.85 mmol) and (*S*)-(1-isothiocyanatoethyl)benzene (465 mg, 2.85 mmol). Purification was carried out by column chromatography using silica gel (CH_2Cl_2/MeOH = 8:2) to afford the title compound **10** (600 mg, 62%) as an off-white solid (Mp: 159-160 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.23 (bs, 1H), 9.92 (bs, 1H), 8.21 (bs, 1H), 7.44 – 7.28 (m, 7H), 7.16-7.14 (m, 2H), 5.53 (p, J = 6.9 Hz, 1H), 1.69 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 153.8, 153.1 (2C), 142.3 (2C), 128.9 (2C), 127.7, 126.4 (2C), 121.9 (2C), 55.1, 21.9.

4.2.7. (R)-N-[N-(1H-benzimidazol-2-yl)carbamimidoyl]-N'-[1-phenylethyl]thiourea (**11**)

Prepared according to the synthesis procedure of **3** using 1-(1*H*-benzo[*d*]imidazol-2-yl)guanidine (200 mg, 1.14 mmol) and (*R*)-(1-isothiocyanatoethyl)benzene (186 mg, 1.14 mmol). Purification was carried out by column chromatography using silica gel (CH_2Cl_2/MeOH = 8:2) to afford the title compound **11** (235 mg, 60%) as an off-white solid (Mp: 159-160 °C). *R*-thiourea **11** has similar experimental and spectral data with *S*-thiourea **10**.

4.2.8. (S)-2-Amino-N-(1-phenylethyl)-1H-benzo[d]imidazole-1-carboxamide (**13**)

To a stirred solution of 1*H*-benzo[*d*]imidazol-2-amine (500 mg, 3.76 mmol) in DMF (10 mL) at 0 °C, was added (*S*)-(1-isocyanatoethyl)benzene (552 mg, 3.76 mmol) and stirred at the

same temperature for 6 h. After the reaction is over (monitored by TLC) water (30 mL) was added and the mixture extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/ EtOAc = 7:3) to give compound **13** as an off-white solid (790 mg, 75%) (Mp: 109-110 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.32 (m, 5H), 7.27-7.25 (m, 2H), 7.23-7.19 (m, 1H), 7.09 – 7.04 (m, 1H), 6.17 (bs, 2H), 6.12 (bs, 1H), 5.20 (p, J = 6.9 Hz, 1H), 1.69 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.3, 151.4, 143.0, 142.0, 129.8, 129.1, 128.0, 126.1, 124.2, 120.5, 117.3, 110.0, 50.9, 22.0. HPLC (OJ column; hexane/2-propanol = 75/25; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(+) = 11.2 min. Enantiomeric rate: (+) = 100.0 and (-) = 0.0.

4.2.9. tert-Butyl 2-amino-1H-benzo[d]imidazole-1-carboxylate (**14**)

To a stirred solution of 1*H*-benzo[*d*]imidazol-2-amine (500 mg, 3.76 mmol) in DMF (10 mL) Et_3N (0.5 mL, 3.76 mmol) was added to the solution, followed by di-*tert*-butyl dicarbonate (820 mg, 3.76 mmol). The reaction was stirred for 6 h at room temperature. The reaction mixture was quenched with water and then acidified with citric acid (pH=3), and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/ EtOAc = 8:2) to give compound **14** as a light yellow solid (750 mg, 85%) (Mp: 155-156 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.19 (t, J = 7.8 Hz, 1H), 7.05 (t, J = 7.8 Hz, 1H), 6.81 (bs, 2H), 1.71 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2, 150.7, 142.3, 130.3, 124.3, 120.6, 116.2, 113.9, 85.8, 28.1.

4.2.10. tert-Butyl (S)-2-(3-(1-phenylethyl)ureido)-1H-benzo[d]imidazole-1-carboxylate (**15**)

To a stirred solution of *tert*-butyl 2-amino-1*H*-benzo[*d*]imidazole-1-carboxylate (750 mg, 3.22 mmol) in DMF (10 mL) was added (*S*)-(1-isocyanatoethyl)benzene (473 mg, 3.22 mmol) and stirred at room temperature for 48 h. After the reaction is over (monitored by TLC) water (20 mL) was added and the mixture extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/ EtOAc = 7:3) to give compound 15 as an off-white solid (900 mg, 73%).^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (d, J = 7.7 Hz, 1H), 9.52 (br, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.48 (d, J = 7.7 Hz, 1H), 7.43 (d, J = 7.7 Hz, 2H), 7.37-7.33 (m, 2H), 7.31 – 7.15 (m, 3H), 5.17 (p, J = 7.0 Hz, 1H), 1.71 (s, 9H), 1.62 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2, 150.1, 148.2, 143.9, 140.2, 129.4, 128.6, 127.1, 126.0, 124.8, 122.9, 117.8, 114.4, 87.4, 50.0, 28.0, 23.2.

4.2.11. (S)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1-phenylethyl)urea (**16**)

The *N*-Boc-derivative **15** (900 mg, 3.22 mmol) was dissolved in dichloromethane (10 mL) and then was added TFA (0.5 mL). The reaction mixture was stirred for 4h at ambient temperature, solvent was removed under reduced pressure. The residue was dissolved in EtOAc (40 mL) and washed with saturated $NaHCO_3$ (20 mL) and brine. The combined organic layer was dried over Na_2SO_4 and the solvent was evaporated to afford pure compound as an off-white solid (500 mg, 75%) (Mp: 86-87 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.81 (bs, 2H), 7.35 – 7.18 (m, 7H), 7.12 – 7.04 (m, 2H), 6.03 (bs, 1H), 5.00 (p, J = 7.0 Hz, 1H), 1.48 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9 (2C), 149.7, 143.5 (2C), 128.7 (2C), 127.4 (2C), 125.9 (2C), 121.8, 49.9, 22.6. HPLC (OJ column; hexane/2-propanol = 80/20; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 8.4 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

4.2.12. (S)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1-phenylethyl)thiourea (**17**)

To a stirred solution of 1*H*-benzo[*d*]imidazol-2-amine (500 mg, 3.76 mmol) in DMSO (10 mL) was added (*S*)-(1-isothiocyanatoethyl)benzene (613 mg, 3.76 mmol) and stirred at 100 °C for 12 h. The reaction mixture cooled to room temperature, water was added and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/ EtOAc = 7:3) to give compound **17** as a white solid (780 mg, 70%) (Mp: 186-187 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.94 (br, 2H), 7.33-7.25 (m, 5H), 7.2-7.19 (m, 2H), 7.1-7.06 (m, 2H), 5.99 (br, 1H), 4.9 (p, J = 7.0 Hz, 1H), 1.47 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.9 (2C), 149.8, 143.4 (2C), 128.8 (2C), 127.5(2C), 126.0(2C), 121.9, 50.0, 22.5. HPLC (OJ column; hexane/2-propanol = 70/30; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(+) = 5.6 min. Enantiomeric rate: (+) = 100.0 and (-) = 0.0.

4.2.13. (R)-1-(1H-Benzo[d]imidazol-2-yl)-3-(1-phenylethyl)thiourea (**18**)

Prepared according to the synthesis procedure of **17** using 1*H*-benzo[*d*]imidazol-2-amine (200 mg, 1.50 mmol) and (*R*)-(1-isothiocyanatoethyl)benzene (245 mg, 1.50 mmol). Purification was carried out by column chromatography using silica gel (Hexane/ EtOAc = 8:2) to afford the title compound **18** (312 mg, 70%) as an off-white solid (Mp: 186-187 °C). *R*-18 has similar experimental and spectral data with *S*-17.

4.2.14. (S)-1-(1H-Indol-5-yl)-3-(1-phenylethyl)urea (**19**)

To a stirred solution of (*S*)-(1-isocyanatoethyl)benzene (113.3 mg, 0.75 mmol) in CH_2Cl_2 (10 mL) was added 1*H*-indol-5-amine (100 mg, 0.84 mmol) and stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was recrystallized from CH_2Cl_2/MeOH to give compound **19** as a light brown solid (200 mg, 95%) (Mp: 216.5-217.5 °C). ^{1}H NMR (400 MHz, MeOD) δ 7.52 (d, J = 2.4 Hz, 1H), 7.41 – 7.27 (m, 6H), 7.27 – 7.16 (m, 2H), 7.02 (dd, J = 8.7, 2.0 Hz, 1H), 6.38 (d, J = 2.4 Hz, 1H), 5.02 – 4.79 (m, 1H), 1.46 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 158.6, 146.3, 134.8, 134.8, 131.9, 129.6, 128.0, 126.9, 126.4, 117.8, 113.8, 112.2, 102.3, 50.7, 23.5.

4.2.15. (S)-1-(1H-Indol-5-yl)-3-(1-phenylethyl)thiourea (**20**)

Prepared according to the synthesis procedure of **19** using 1*H*-indol-5-amine (100 mg, 0.75 mmol) and (*S*)-(1-isothiocyanatoethyl)benzene (124 mg, 0.75 mmol). Purification was carried out by column chromatography using silica gel (Hexane/ EtOAc = 8:2) to afford the title compound **20** (205 mg, 92%) as a light brown solid (Mp: 94.5-95.5 °C). ^{1}H NMR (400 MHz, MeOD) δ 7.36 – 7.26 (m, 2H), 7.25 – 7.15 (m, 5H), 7.15 – 7.04 (m, 1H), 6.85 (dd, J = 8.5, 1.9 Hz, 1H), 6.34 (d, J = 2.8 Hz, 1H), 5.60 – 5.49 (m, 1H), 1.33 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, MeOD) δ 181.8, 144.8 (2C), 136.5, 130.0, 129.5, 128.1, 127.3 (2C), 121.0, 119.0, 113.1, 102.8, 55.1, 22.0.

4.2.16. Benzyl (1H-indol-3-yl)carbamate (**26**)

Diphenylphosphoryl azide (3.21 mL, 14.89 mmol) and Et_3N (2.08 mL, 12.89 mmol) were added to a stirred solution of 1*H*-indole-3-carboxylic acid (2.0 g, 12.41 mmol) in benzene (40 mL). After the mixture was stirred at room temperature for 24 h, benzyl alcohol (3. 86 mL, 37.23 mmol) was added to the reaction mixture and refluxed for 48 h. After the mixture was cooled to room temperature, the solvent was evaporated. The residue was diluted with EtOAc and washed with water (3 × 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/ EtOAc = 9:1) to give compound 26 as an off-white solid (2.5

g, 80%) (Mp: 165-166 °C). ^{1}H-NMR (400 MHz, Aceton-d_6): δ 9.96 (bs, 1H), 8.54 (bs, 1H), 7.79 (d, J = 7.5 Hz, 1H), 7.61 (bs, 1H), 7.50-7.32 (m, 6H), 7.13 (t, J = 7.5 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 5.21 (s, 2H). ^{13}C-NMR (100 MHz, Aceton-d_6): δ 154.3, 137.6, 134.8, 128.6, 128.2, 128.1, 122.2, 121.4, 118.6, 117.8, 115.8, 114.6, 111.5, 66.2.

*4.2.17. 1H-Indol-3-amine (**27**)*

N-(1*H*-Indol-3-yl)-2-phenylacetamide (1.0 g, 3.76 mmol) was dissolved in MeOH (30 mL) and 10% Pd/C (100 mg) was added. The mixture was stirred in an H_2 atmosphere for 4 h. The Pd/C was removed by filtration and concentrated under reduced pressure to afford 1*H*-indol-3-amine which was used in the next step without further purification.

*4.2.18. (S)-1-(1H-Indol-3-yl)-3-(1-phenylethyl)urea (**21**)*

To a stirred solution of 1*H*-indol-3-amine (400 mg, 3.03 mmol) in CH_2Cl_2 (10 mL) Et_3N (0.42 mL, 3.03 mmol) was added to the solution, followed by (*S*)-(1-isocyanatoethyl)benzene (445 mg, 3.03 mmol). The reaction was stirred for 12 h at room temperature. The mixture was washed with water and brine, combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography respectively (hexane/ EtOAc = 8:2) and (MeOH, 150 mL) to obtain pure product (**21**) as a light-green oil (600 mg, 71%).^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.53 (bs, 1H), 7.45 (d, J = 7.4 Hz, 1H), 7.29-7.12 (m, 7H), 7.09-7.05 (m, 2H), 6.45 (bs, 1H), 5.31 (bs, 1H), 5.01 (p, J = 7.0 Hz, 1H), 1.33 (d, J = 7.0 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 157.1, 144.1, 134.8, 128.6, 127.1, 125.9 (2C), 123.5, 122.8, 120.1, 117.7, 114.1, 111.7, 49.7, 22.7. HPLC (OJ column; hexane/2-propanol = 80/20; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 19.1 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

*4.2.19. (S)-1-(1H-Indol-3-yl)-3-(1-phenylethyl)thiourea (**22**)*

Prepared according to the synthesis procedure of **21** using 1*H*-indol-3-amine (400 mg, 3.03 mmol), Et_3N (0.42 mL, 3.03 mmol) and (*S*)-(1-isothiocyanatoethyl)benzene (494 mg, 3.03 mmol). Purification was carried out by column chromatography using respectively (hexane/ EtOAc = 8:2) and (MeOH, 150 mL) to obtain pure product (**26**) as a light-green oil (620 mg, 73%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.52 (bs, 1H), 7.64 (bs, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.28-7.07 (m, 8H), 6.22 (d, J = 5.5 Hz, 1H), 5.73-5.70 (m, 1H), 1.42 (d, J = 6.8 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 181.1, 142.5, 135.0, 128.6, 127.4, 126.2, 123.5, 123.3, 121.3, 121.0, 118.0, 112.4, 112.0, 54.1, 21.5. HPLC (OJ column; hexane/2-propanol = 80/20; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 30.3 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

*4.2.20. Benzyl (1H-indol-2-yl)carbamate (**29**)*

Prepared according to the synthesis procedure of **26** using diphenylphosphoryl azide (3.21 mL, 14.89 mmol), Et_3N (2.08 mL, 12.89 mmol) and 1*H*-indole-2-carboxylic acid (2.0 g, 12.41 mmol). Purification was carried out by column chromatography using silica gel (hexane/ EtOAc = 9:1) to afford the title compound **29** (2.4 g, 72%) as an off-white solid (Mp: 141-142 °C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.80 (bs, 1H), 7.47-7.33 (m, 6H), 7.31-7.27 (m, 1H), 7.15 (bs, 1H), 7.12-7.05 (m, 2H), 5.82 (s, 1H), 5.24 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 153.4, 135.4, 134.0, 132.5, 128.7, 128.6, 128.3, 127.2, 120.5, 120.2, 119.0, 110.6, 85.1, 67.7.

*4.2.21. 1H-Indole-2-carbonyl chloride (**32**)*

To a stirred solution of indoline-2-carboxylic acid (1.0 g, 6.21 mmol) in CH_2Cl_2 (15 mL) was slowly added $SOCl_2$ (1 mL, 14.2 mmol). The reaction mixture was then refluxed at 70 °C for 2h. The solvent was removed on a rotary evaporator. The crude product was purified by column chromatography (hexane/ EtOAc = 9:1) to give compound **32** as a light-yellow solid (1.05 g, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.99 (bs, 1H), 7.75-7.73 (m, 1H), 7.55-7.54 (m, 1H), 7.44-7.43 (m, 2H), 7.20 (m, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 160.0, 138.5, 129.4, 127.9, 127.0, 123.5, 121.8, 116.2, 112.3.

*4.2.22. 1H-Indole-2-carbonyl azide (**33**)*

To a solution of 1*H*-indole-2-carbonyl chloride (1.05 g, 5.85 mmol) in THF (15 mL) a solution of NaN_3 (2.28 g, 35.08 mmol) in H_2O (3 mL) was added. Upon completion of the addition, the resulting mixture was stirred for 48 at room temperature. Water (20 mL) was added and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over Na_2SO_4. Removal of the solvent under reduced pressure gave **33**, which was used without further purification in the next step (1.0 g, 91%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.99 (bs, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 7.37 (t, J = 7.9 Hz, 1H), 7.29 (s, 1H), 7.17 (t, J = 7.9 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): 166.3, 138.1, 128.1, 127.3, 126.7, 123.1, 121.3, 112.1, 111.0.

*4.2.23. (R)-N-(1-Phenylethyl)-1H-indole-2-carboxamide (**35**)*

To a stirred solution of 1*H*-indole-2-carbonyl chloride (500 mg, 2.78 mmol) in CH_2Cl_2 (10 mL) was added (*R*)-1-phenylethan-1-amine (337 mg, 2.78 mmol) and stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane/ EtOAc = 7:3) to give compound **35** (660 mg, 90%) as a light-yellow solid (Mp: 167-168 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.85 (bs, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.44-7.34 (m, 4H), 7.33-7.26 (m, 2H), 7.26-7.20 (m, 1H), 7.14-7.07 (m, 1H), 6.86 (s, 1H), 6.45 (d, J = 7.1 Hz, 1H), 5.39 (p, J = 7.1 Hz, 1H), 1.64 (d, J = 7.1 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 161.0, 143.0, 136.5, 130.6, 128.8, 127.6, 127.5, 126.2, 124.5, 121.8, 120.6, 112.12, 102.0, 49.1, 22.0. HPLC (OJ column; hexane/2-propanol = 90/10; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 52.4 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

*4.2.24. 2,2,2-Trifluoro-N-(2-iodophenyl)acetamide (**37**)*

To a solution of 2-iodoaniline (1.0 g, 4.30 mmol) and triethylamine (0.7 mL, 4.55 mmol) in THF (10 mL) at -15 °C was slowly added trifluoroacetic anhydride (0.65 mL, 4.30 mmol) in 5 mL of THF. The resulting mixture was stirred for 1 h and then allowed to warm to room temperature and stirred for 16 h. The reaction mixture was then poured into a separatory funnel containing water (100 mL) and extracted with ethyl acetate (3 × 30 mL). The organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give desired product **37** (1.3 g, 90%) as a white solid (Mp: 105-106 °C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 8.21 (d, J = 7.6 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 6.98 (t, J = 7.6 Hz, 1H) [34].

4.2.25. 2-Amino-1H-indole-3-carbonitrile (38)

To a stirred solution of 2,2,2-trifluoro-*N*-(2-iodophenyl)acetamide (1.3 g, 0.5 mmol) in DMSO (15) was added malononitrile (1.3 g, 0.5 mmol), L-proline (95 mg, 0.1 mmol) and K_2CO_3 (1.14 g, 1.0 mmol). After stirring the mixture for 15 min under nitrogen atmosphere CuI (78 mg, 0.05 mmol) was added to the flask. The mixture was stirred at 60 °C for 12 h. The resulting mixture was cooled to room temperature and filtered. The solid was washed with methanol two times (2 × 3 mL), and the combined filtrate was concentrated on the rotary evaporator, and the residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (1:2) as eluent to give the desired product **38** (1.1 g, 85%) as a light yellow solid (Mp: 191-192 °C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H), 7.13 (d, J = 8.3 Hz, 2H), 6.30 (m, 2H), 6.70 (s, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 154.3, 132.6, 128.8, 121.2, 120.1, 118.4, 115.6, 110.7, 62.2 [35].

*4.2.26. (S)-1-(3-Cyano-1H-indol-2-yl)-3-(1-phenylethyl)urea (**39**)*

To a stirred solution of 2-amino-1*H*-indole-3-carbonitrile (1.0 g, 6.36 mmol) in THF (10 mL) NaH (55-60%) (254 mg, 6.36 mmol) was added and after stirring of the mixture for 15 min, (*S*)-(1-isocyanatoethyl)benzene (936 mg, 6.36 mmol) was added to reaction mixture. The mixture was stirred at room temperature for 12 h. Solvent was evaporated and the residue was diluted with EtOAc and washed with water (3×20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give desired product **39** (500 mg, 25%) as a red solid (Mp: 174-175 °C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.87 (s, 1H), 8.93 (s, 1H), 7.49-7.37 (m, 5H), 7.37-7.11 (m, 4H), 6.33 (d, $J = 7.0$ Hz, 1H), 5.00 (p, $J = 7.0$ Hz, 1H), 1.60 (d, $J = 7.0$ Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 153.9, 147.0, 143.5, 131.4, 128.8, 127.5, 125.8, 125.4, 122.3, 122.2, 118.0, 117.4, 111.5, 65.7, 50.2, 22.9. HPLC (OJ column; hexane/2-propanol = 80/20; flow rate 1.0 mL/ min; T=25 °C; 254 nm): t_R-(-) = 1.0 min. Enantiomeric rate: (+) = 0.0 and (-) = 100.0.

4.3. Antibacterial assay

The antibacterial activity of synthesized compounds was individually tested against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus cereus* ATCC 33019, and *Staphylococcus aureus* ATCC 29213. Microorganisms were cultured using Nutrient agar for 24 h at 37°C. The compounds were dissolved with 15% DMSO to prepare stock solutions of concentration 2000 µg/mL.

4.3.1. Disk diffusion assay

The antibacterial activity of the synthesized compounds was screened with the disk diffusion assay [43]. A hundred µL of bacteria suspensions (containing 10^8 colony-forming-unit/mL bacteria) were spread on the nutrient agar plate. The disks (6 mm in diameter) were placed on the plate and impregnated with 10 µL of the solution (300 µg the compound per disk). DMSO was used as a negative control. Ampicillin (10 µg per disk) was used as a positive control. The plates were incubated for 24 h at 37°C. The antibacterial activity was determined by measuring the diameter of the inhibition zone. The assays were performed three times.

4.3.2. Microwell dilution assay

Minimum inhibitory concentration (MIC) values of the compounds forming the inhibition zone against microorganisms were determined using a microwell dilution assay [44]. The solutions of microorganisms were prepared using bacteria cultures incubated for 12 h and adjusted to 0.5 McFarland standard turbidity. The compounds with different concentrations (ranging from 7.8 to 500 µg/mL) were prepared from the stock solution with 2-fold serial dilutions. For the assay, 5 µL of bacteria solution, 95 µL of Tryptic Soy Broth, and 100 µL of the compound solution were added to each well. The 96-well plates were incubated at 37°C for 24 h. Chloramphenicol at a concentration ranged from 7.8 to 500 µg/mL was used as standard antibacterial agents for positive control. The wells containing only microorganism solution and Tryptic Soy Broth were used as a negative control. After incubated, microbial growth in Tryptic Soy Broth was monitored at 600 nm by the microplate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). To confirm the growth, the samples in clear wells were inoculated on nutrient agar.

4.4. Molecular docking modeling

Molecular modeling was done using the Schrödinger Maestro software package. The molecules (**9**, **16**, and **20**) were prepared using the LigPrep module. The crystal structures were downloaded from the Protein Database (PDB: 3VMR, 3VSL) for molecular docking studies [40,41]. The biomolecule structures were pretreated using the Protein Preparation Wizard to optimize the hydrogen bonding network and remove possible crystallographic artifacts. While Glide XP was used for 3VMR protein structure, the Schrödinger CovDock algorithm used both Glide and Prime were used for 3VSL protein. The original ligand (cefotaxime) of the 3VSL protein structure is covalently bound to Ser 392, and as a result, the connection between the ligand and Ser 392 is removed before minimization. Molecular docking studies were performed using the covalent docking protocol.

4.5. In silico *ADME*

Some ADME properties (QpLogPo/w, QPLogHERG, QPPCaco, QpLogBB, QPPMDCK, QPlogKp, QpLogKksa, Percent Human oral absorption, and Lipinski's five rules) for synthesized compounds (8-13, 16-18, 20-21, and 39) were determined by computational methods using Qikprop.

Author contributions

F.L. synthesized and chemically characterized the compounds. D.K. and M.Y. performed the molecular docking and antimicrobial studies, respectively. N.S. designed and supervised the research study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Research Fund of the Ataturk University; Project Numbers: 2012-146, FBA-2020-8419 and the Scientific and Technological Research Council of Turkey (TÜBİTAK); Project No: 111T998

References

[1] A.R. Jennings, D.Y. Son, Tetrahedron 71 (2015) 3990–3999.
[2] N.K. Rana, R. Unhale, V.K. Singh, Tetrahedron Lett. 53 (2012) 2121–2124.
[3] F. Lafzi, H. Kilic, G. Tanriver, Ö.N. Avcı, S. Catak, N. Saracoglu, Synth. Commun. 49 (2019) 3510–3527.
[4] M.C. Gimeno, R.P. Herrera, Cryst. Growth Des. 16 (9) (2016) 5091–5099.
[5] M.-O.M. Piepenbrock, G.O. Lloyd, N. Clarke, J.W. Steed, Chem. Commun. 23 (2008) 2644–2646.
[6] M.-O.M. Piepenbrock, G.O. Lloyd, N. Clarke, J.W. Steed, Chem. Rev. 110 (2009) 1960–2004.
[7] S.V. Ryabukhin, A.S. Plaskon, E.N. Ostapchuk, D.M. Volochnyuk, A.A. Tolmachev, Synth. (2007) 417–427 2007.
[8] T.K. Venkatachalam, C. Mao, F.M. Uckun, Bioorg. Med. Chem. 12 (2004) 4275–4284.
[9] J. Lee, S.-U. Kang, H.-K. Choi, J. Lee, J.-O. Lim, M.-J. Kil, M.-K. Jin, K.-P. Kim, J.-H. Sung, S.-J. Chung, H.-J. Ha, Y.-H. Kim, L.V. Pearce, R. Tran, D.J. Lundberg, Y. Wang, P.M.Blumberg A.Toth, Bioorg. Med. Chem. Lett. 14 (2004) 2291–2297.
[10] S.A. Khan, N. Singh, K. Saleem, Eur. J. Med. Chem. 43 (2008) 2272–2277.
[11] A.P. Keche, G.D. Hatnapure, R.H. Tale, A.H. Rodge, S.S. Birajdar, V.M. Kamble, Bioorg. Med. Chem. Lett. 22 (2012) 3445–3448.
[12] S. Saeed, N. Rashid, P.G. Jones, M. Ali, R. Hussain, Eur. J. Med. Chem. 45 (2010) 1323–1331.
[13] R. Pingaew, N. Sinthupoom, P. Mandi, V. Prachayasittikul, R. Cherdtrakulkiat, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Med. Chem. Res. 26 (2017) 3136–3148.
[14] K.M. Khan, S. Saeed, M. Ali, M. Gohar, J. Zahid, A. Khan, S. Perveen, M.I. Choudhary, Bioorg. Med. Chem. 17 (2009) 2447–2451.
[15] S. Abuzar, S. Sharma, Indian J. Chem. Sect B. 20 (1981) 230–233.
[16] A. Piskac, I. Fejtova, Veterinarni medicina 25 (1980) 739–742.
[17] A. Ricci, C. Bertoletti. Plant Biol. 11 (2009) 262–272.
[18] N. Ulusoy, E. Gursu, S. Ozkirimli, A.C. Ekinci, I. Dikici, Indian J. Chem. 42B (2003) 2853–2857.

[19] K. Ramadas, G. Suresh, N. Janarthanan, S. Masilamani, Pestic. Sci. 52 (1998) 145–151.
[20] C. Esteva-Font, P.-W. Phuan, S. Lee, T. Su, M.O. Anderson, A.S. Verkman, Biochim. Biophys. Acta 1848 (2015) 1075–1080.
[21] V. Ravichandran, S. Shalini, K.S. Kumar, H. Rajak, R.K. Agrawal, Lett. Drug Des. Discovery. 16 (2019) 618–624.
[22] S. Karakus, B. Koçyığıt-Kaymakcioğlu, H.Z. Toklu, F. Aricioglu, S. Rollas, Arch. Pharm. Chem. Life Sci. 342 (2009) 48–53.
[23] S. Mehrotra, P. Roychowdhury, K.K. Pandey, P.K. Srivastava, Arch. Pharmacal Res. 18 (1995) 356.
[24] J.-Z. Li, S.-J. Xue, Y.-L. Guo. Chin, J. Org. Chem. 27 (2007) 1278–1281.
[25] A.A. Moneer, K.O. Mohammed, H.B. El-Nassan, Chem. Biol. Drug Des. 87 (2016) 784–793.
[26] V. Canale, R. Kurczab, A. Partyka, G. Satała, T. Lenda, M. Jastrzębska-Więsek, A. Wesołowska, A.J. Bojarski, P. Zajdel, Eur. J. Med. Chem. 108 (2016) 334–346.
[27] L. Qiao, S. Hao, Molecules 23 (2018) 600.
[28] A. Mumtaz, A. Majeed, S. Zaib, S.U. Rahman, S. Hameed, A. Saeed, H. Rafique, E. Mughal, A. Maalik, I. Hussain, Bioorg. Chem. 90 (2019) 103036.
[29] D.A. Patrick, T. Wenzler, S. Yang, P.T. Weiser, M.Z. Wang, R. Brun, R.R. Tidwell, Bioorg. Med. Chem. 24 (2016) 2451–2465.
[30] M. Cindric, S. Jambon, A. Harej, S. Depauw, M.H. David-Cordonnier, S.K. Pavelic, G. Karminski-Zamola, M. Hranjec, Eur. J. Med. Chem. 136 (2017) 468–479.
[31] D. Ashok, S. Gundu, V.K. Aamate, M.G. Devulapally, Mol. Diversity. 22 (2018) 769–778.
[32] J.L. Charlton, K. Koh, G.L. Plourde, Can. J. Chem. 68 (1990) 2028–2032.
[33] N. Öztaşkın, S. Göksu, H. Secen, Synth. Commun. 41 (2011) 2017–2024.
[34] Y. Chen, N.A. Markina, R.C. Larock, Tetrahedron 65 (2009) 8908–8915.
[35] X. Yang, H. Fu, R. Qiao, Y. Jiang, Y. Zhao, Adv. Synth. Catal. 352 (2010) 1033–1038.
[36] T.J. Silhavy, D. Kahne, S. Walker, Cold spring harbor perspect, Biol 2 (2010) a000414.
[37] S.I. Miller, MBio 7 (2016) e01541-16.
[38] Y. Ali, N.M. Bunnori, D. Susanti, A.M. Alhassan, S.A. Hamid, Front. Chem. 6 (2018) 210.
[39] A. Saeed, S. Zaib, A. Pervez, A. Mumtaz, M. Shahid, J. Iqbal. Med. Chem. Res. 22 (2013) 3653–3662.
[40] H. Yoshida, F. Kawai, E. Obayashi, S. Akashi, D.I. Roper, J.R. Tame, S.Y. Park, J. Mol. Biol. 423 (2012) 351–364.
[41] C.Y. Huang, H.W. Shih, L.Y. Lin, Y.W. Tien, T.J. Cheng, W.C. Cheng, C.H. Wong, C. Ma, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 6496–6501.
[42] K. Kalani, D.K. Yadav, F. Khan, S.K. Srivastava, N. Suri, J. Mol. Model. 18 (2012) 3389–3413.
[43] F. Ntie-Kang, Springerplus 2 (2013) 353.
[44] B. Cetin, H. Ozer, A. Cakir, T. Polat, A. Dursun, E. Mete, E. Ozturk, M. Ekinci, J. Med. Food. 13 (2010) 196–204.